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# SPATIAL RELATIONSHIPS BETWEEN MARINE BACTERIA AND LOCALIZED CORROSION ON STEELS

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Running title: Spatial Relationships

## INTRODUCTION

The role that bacteria play in producing/influencing anodic and cathodic corrosion reactions has been documented in the literature on microbiologically influenced corrosion (MIC). However, the impact of established anodic and cathodic polarizations on deposition or settlement of bacteria has not been adequately addressed. One of the most serious challenges in diagnosis of MIC is differentiation between biological and abiological processes. Most MIC investigations determine spatial relationships between bacteria and corrosion products using microscopy or microbiological diagnostic kits. Spatial relationships are then interpreted as causal. Results from laboratory and field experiments demonstrate that spatial relationships cannot be interpreted as causal. Anodic reactions/products influence the distribution of marine bacteria on both carbon and stainless steel. The attraction of bacteria to anodic sites does not depend on viability of the organisms or concentration of the electrolyte.

## MATERIALS AND METHODS

### Stainless steel exposures in the laboratory

Experiments were designed using 304 stainless steel electrodes with intentional crevices initiated by placing a small piece of sterile wood under the gasket of the electrode holder across the face of the electrode. Identical electrodes were maintained without crevices. Electrodes were exposed to 700 ml filtered 35 ppt salinity artificial seawater (0.10  $\mu$ m Millipore filter) to allow formation of abiotic corrosion products on specimens with crevices. Open circuit potential measurements ( $E_{\text{corr}}$ ) as a function of time were measured with Princeton EG&G potentiostats (Model 173, Model 273, and Model 350). After 11 days, 50 ml of natural seawater was added. Abiotic controls were maintained for all exposures. After 5 days electrodes were removed, placed in seawater-buffered glutaraldehyde and examined using environmental scanning electron microscopy (ESEM) coupled with energy dispersive x-ray analysis (EDS). ESEM/EDS operating parameters have been described elsewhere (1).

### Polymer-coated mild steel exposures

Triplicate cold rolled steel panels (10.16 cm x 15.24 cm) were coated with the following combinations: zinc primer and epoxy polyamide topcoat; zinc primer, epoxy polyamide midcoat, and polyurethane topcoat; phosphate primer and epoxy polyamide topcoat; phosphate primer, epoxy polyamide midcoat, and polyurethane topcoat; ion vapor-deposited (IVD) aluminum (Al) primer and epoxy polyamide topcoat; IVD-Al primer, epoxy polyamide midcoat, and polyurethane topcoat; and three layers of epoxy polyamide (Table I). Panels were exposed with and without intentional defects. Defects were introduced by drilling four 0.32-cm-diameter holes (two per side) 2.54 cm from the bottom edge and 2.54 cm from the side edge (Figure 1) through the coating to the metal surface



Table I. Composition and Thickness Coating Systems on Cold Rolled Steel

Coating	A	B	C	D	E	F	G
Primer	Zinc <sup>a</sup>	Zinc <sup>a</sup>	Phosphate <sup>d</sup>	Phosphate <sup>d</sup>	IVD-Al <sup>e</sup>	IVD-Al <sup>e</sup>	Epoxy <sup>f</sup>
Midcoat		Epoxy <sup>b</sup>		Epoxy <sup>b</sup>		Epoxy <sup>b</sup>	Epoxy <sup>f</sup>
Topcoat	Epoxy <sup>b</sup>	Polyurethane <sup>c</sup>	Epoxy <sup>b</sup>	Polyurethane <sup>c</sup>	Epoxy <sup>b</sup>	Polyurethane <sup>c</sup>	Epoxy <sup>f</sup>
Ave. Thickness (μm)	53	83	43	76	81	81	81

<sup>a</sup>Zinc plate per QQ-Z-325 Rev. C (Primer Type II, Class2).

<sup>b</sup>2 coats of epoxy polyimide MIL-P-233377 Rev. G (Type I, Class C) per MIL-F-18264 REV. D AMD.1.

<sup>c</sup>1 coat of MIL-C-85285 Rev. B (AS) AMD.AMD.1.(AR)2 polyurethane per MIL-F18264 REV. D AMD.1 color #36375 lusterless grey of FED-STD-595.

<sup>d</sup>Phosphate coat DOD-P-16232 RE. F (Type Z, Class3); hydrogen embrittlement relieved for 8 minimum hours at 375°F ±25°F.

<sup>e</sup>Ion vapor deposited-aluminum per MIL-C-83488-C (Notice 1, Type II, Class 1).

<sup>f</sup>1 coat of MIL-P-24441/2 Rev. B epoxy polyamide.

to create a defined holiday in the coating. Some panels with intentional defects were cathodically protected by attaching a zinc anode; others were allowed to freely corrode.

Panels were exposed for 30 days to natural seawater at the Key West, FL, Naval Research Laboratory Marine Corrosion Facility and to artificial seawater in laboratory tests. At the end of exposure, panels from the natural seawater exposures were shipped to the Naval Research Laboratory, Stennis Space Center, MS (NRLSSC) in closed, Plexiglas<sup>®</sup> containers filled with natural seawater. Containers were constructed with slots so that multiple panels were secured in the orientation in which they had been exposed. Plexiglas<sup>®</sup> containers were shipped by overnight courier in insulated cartons with ice packs. Panels were transferred to separate containers of 4% glutaraldehyde in 0.45 μm filtered natural seawater and refrigerated overnight. Wet panels were photographed and examined with a Wild Heerbrugg M8 zoom stereo microscope. Prior to microscopic examination, panels were removed from glutaraldehyde fixative and gently rinsed in distilled water. After photography areas of interest were carefully cut from wet panels for ESEM examination.

Electrochemical noise (EN) and impedance (EIS) data were measured using a two-electrode arrangement for simultaneous collection of potential and current noise. Data collection was performed remotely via computer control twice a week. The time dependence of the potential of two coupled electrodes ( $E_{\text{coup}}$ ), coupling current ( $I_{\text{coup}}$ ), noise resistance ( $R_n$ ), and spectral noise resistance ( $R_{\text{sn}}^0$ ) was determined from EN data.  $R_n$  was defined as the ratio of the standard deviations of the potential noise ( $\sigma\{V(t)\}$ ) and the current noise ( $\sigma\{I(t)\}$ ) as follows:

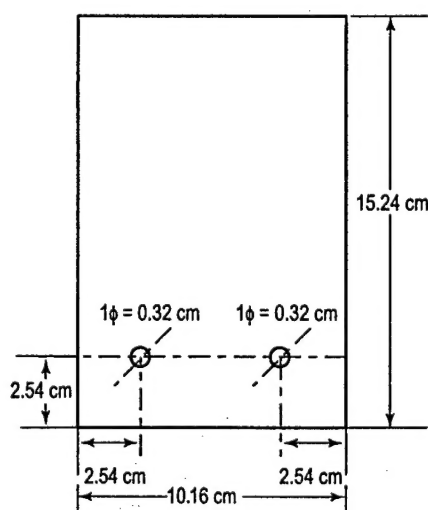


Figure 1. Schematic of field exposure panels with intentional defects.



$(R_n) = \sigma\{V(t)\}/\sigma\{I(t)\}$ .  $R_{sn}^0 = \lim \{R_{sn}(f)\}$  for  $f \rightarrow 0$ , where  $R_{sn}(f)$  is spectral noise and  $f$  is frequency of potential or current fluctuations (2). Spectral noise plots can be determined from power spectral density (PSD) plots for potential and current fluctuations and used to evaluate coating degradation (3). Experimental  $R_n$  values can depend on the bandwidth  $\Delta f$  of the EN (4). In those cases where no dc limit was observed in the spectral noise plots,  $R_{sn}^0$  values were calculated using the average of the last 10 data points (2,3). Decreasing  $R_n$  and  $R_{sn}^0$  values are indicative of a degrading coating system.

### Spatial Relationship Experiments

Iron filings (1 gram) were allowed to corrode overnight in individual drops of sterilized natural seawater from Key West, basal salts medium and distilled water. A 20  $\mu$ l aliquot of a mixed culture grown to log phase from natural seawater was added to each drop. Duplicate aliquots were fixed in glutaraldehyde 4 hours prior to addition. After one hour, 10  $\mu$ l were removed, fixed in glutaraldehyde and examined with a Molecular Dynamics Sarastro 2000 confocal laser scanning microscope (CLSM).

## RESULTS

### Stainless steel

Corrosion products on 304 stainless steel in which an abiotic crevice was first induced were associated with bacteria after a 5-day exposure to natural seawater (Figure 2a). Within the corrosion products spirilla, rods, vibrio and filamentous cells were identified (Figure 2b). Cell types tended to be isolated from one another and did not appear in consortia as one would expect if the surface had indeed been colonized by marine bacteria. The remainder of the electrode surface was free of corrosion and only isolated bacterial cells could be located. In abiotic controls corrosion products formed in an identical fashion. In all cases, the corrosion products were enhanced in chromium relative to the base alloy. The presence of bacteria did not change the distribution or elemental composition of the corrosion products.

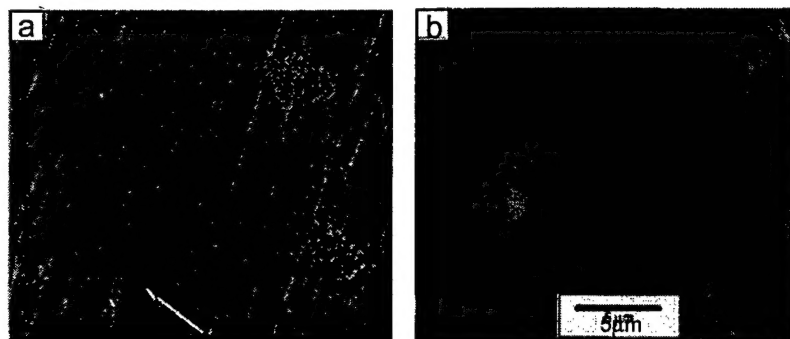


Figure 2. (a) Abiotically-formed corrosion products exposed to marine bacteria (40x). (b) Bacteria enmeshed with corrosion products.

### Polymer-coated mild steel exposures

Metallic zinc and IVD-Al primer (coatings A, B, E, and F) provided cathodic protection for coatings without intentional defects. After 30 days, coatings were discolored due to the presence of microalgae and macrofouling, but there was no localized corrosion. Localized corrosion was prominent on surfaces with phosphate primer and the all-epoxy polyamide coatings (coatings C, D, and G). In all cases, delamination was accompanied by localized corrosion and accumulation of bacteria (Figure 3a & b). In the presence of cathodic protection by an external zinc

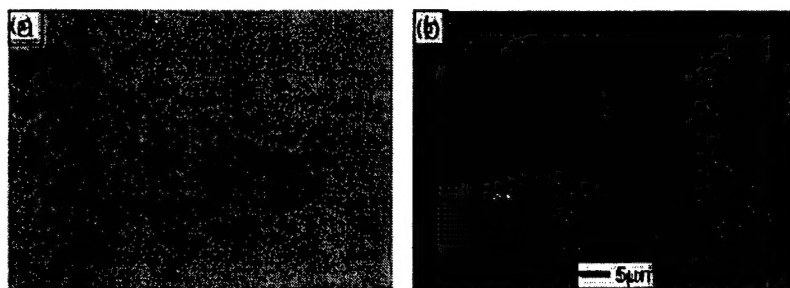


Figure 3. (a) Localized corrosion on panel coated with phosphate primer and epoxy polyamide topcoat (coating C, 6x) and (b) bacteria associated with corrosion.



anode, intentional defects were filled with calcareous deposits. EDS spectra of calcareous deposits indicate the presence of zinc and phosphate from the primer in addition to calcium. There were no indications of corrosion and the numbers of individual bacterial cells within the defects were similar to the numbers of cells on the intact coating. There were no accumulations of bacteria within any of the defects protected by an external anode.

Coating composition influenced corrosion behavior and location of bacteria in defects without cathodic protection from zinc anodes.  $E_{\text{coup}}$  values for coatings with metallic zinc primer were close to  $-1000$  mV vs. Ag/AgCl (Figure 4) similar to open circuit potential ( $E_{\text{corr}}$ ) of zinc in seawater.  $E_{\text{coup}}$  values for all other samples were approximately  $-700$  mV, close to  $E_{\text{corr}}$  for iron in seawater.

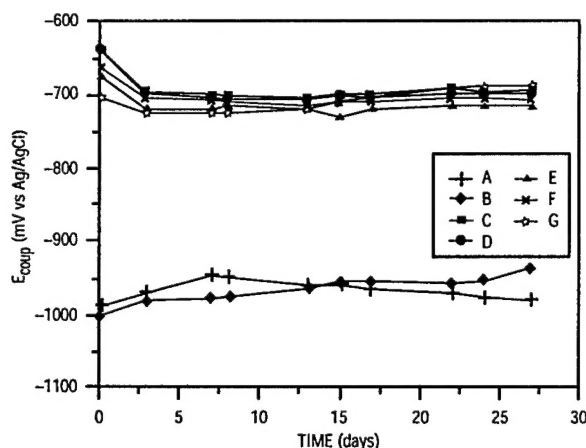


Figure 4. Time dependence of  $E_{\text{coup}}$  for coatings with defects exposed in Key West, FL. Letter designations refer to the coatings in Table I.

Defects in the coatings with zinc primer were, in all cases, filled with calcareous deposits and no corrosion products or accumulations of bacteria were observed. While the zinc primer on steel provided cathodic protection to exposed steel in defects, the same effect was not consistently observed for samples with IVD-Al primer. One of the four panels with IVD-Al primer had no indication of corrosion products in any of the defects, while all others were filled with corrosion. When corrosion was present, there were accumulations of bacterial cells.

In the absence of cathodic protection provided by an external anode or the primer, most defects in the other coatings were filled with voluminous corrosion products (Figure 5a-c) and large accumulations of bacteria (Figure 6a-c). ESEM images were collected from the center of the corroding defect. Large accumulations of bacteria could only be located in association with corrosion products. Even though the specimens were exposed to the same body of seawater, there were morphological differences among the bacteria within defects of various coatings.

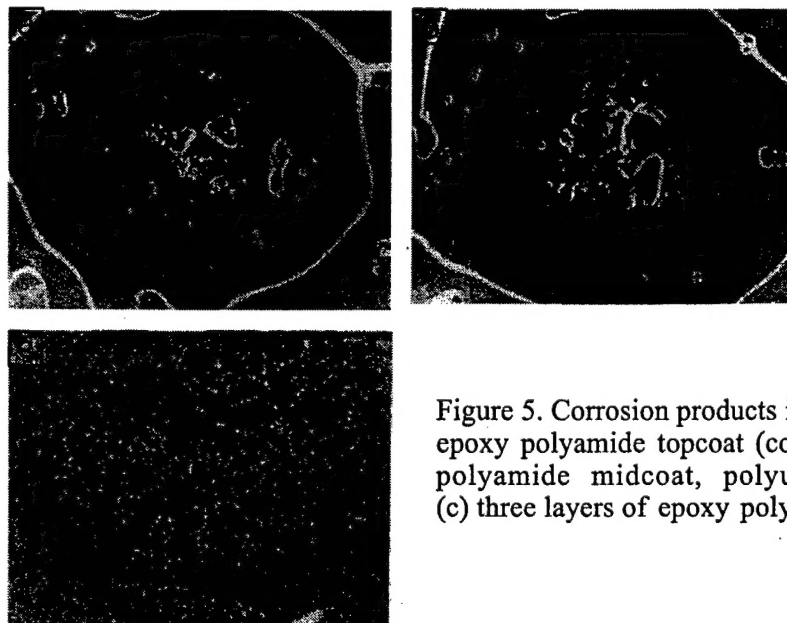


Figure 5. Corrosion products in coating defects, (a) phosphate primer, epoxy polyamide topcoat (coating C), (b) phosphate primer, epoxy polyamide midcoat, polyurethane topcoat (coating D), and (c) three layers of epoxy polyamide (coating G) (6x).



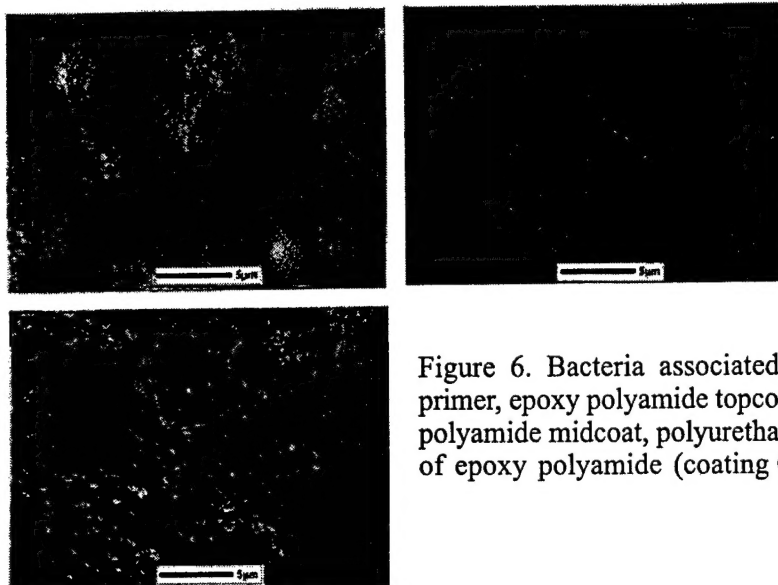


Figure 6. Bacteria associated with corrosion products, (a) phosphate primer, epoxy polyamide topcoat (coating C), (b) phosphate primer, epoxy polyamide midcoat, polyurethane topcoat (coating D), and (c) three layers of epoxy polyamide (coating G).

$I_{\text{coup}}$  values decreased with time for coatings with zinc primer (Figure 7a), while for the other samples,  $I_{\text{coup}}$  remained relatively constant after 7 days immersion (Figure 7b,c). Small changes were observed in  $R_{\text{sn}}^0$  values for coatings with zinc primer increased (Figure 8a), while  $R_{\text{sn}}^0$  for coatings with phosphate primers decreased with exposure time (Figure 8b). No significant changes were found for other samples (Figure 8c).

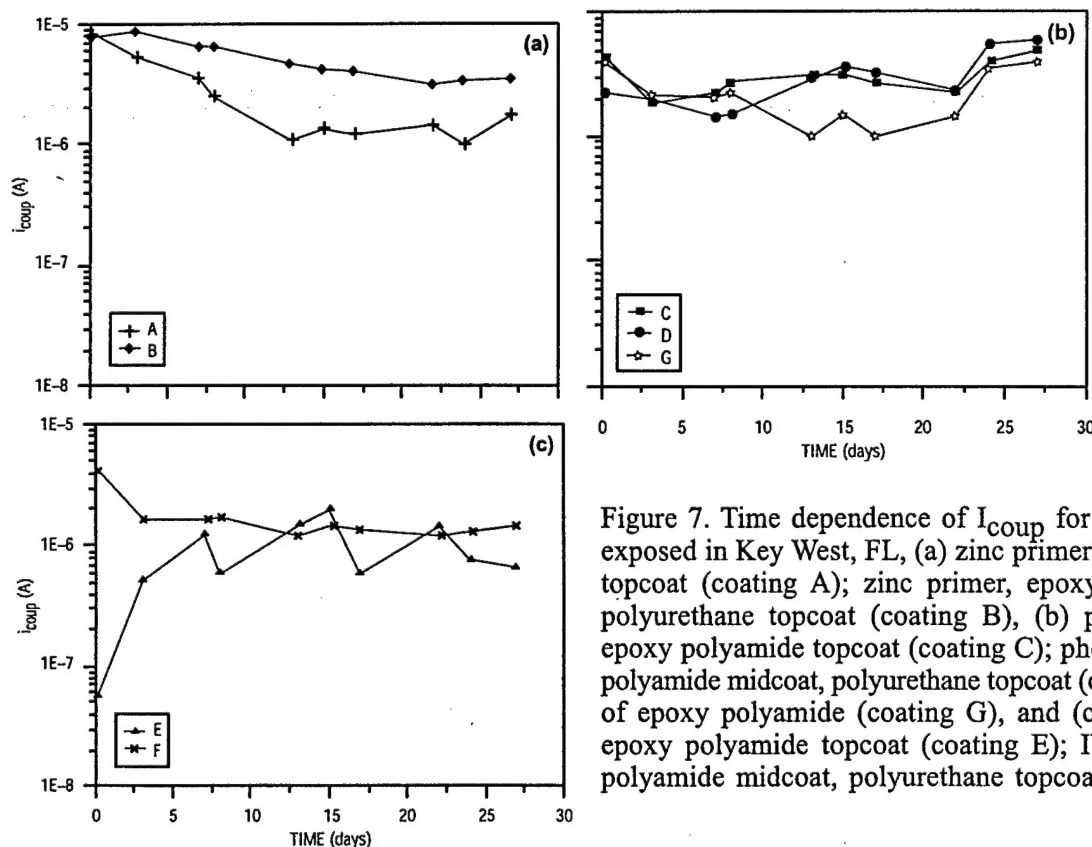


Figure 7. Time dependence of  $I_{\text{coup}}$  for samples with defects exposed in Key West, FL, (a) zinc primer and epoxy polyamide topcoat (coating A); zinc primer, epoxy polyamide midcoat, polyurethane topcoat (coating B), (b) phosphate primer and epoxy polyamide topcoat (coating C); phosphate primer, epoxy polyamide midcoat, polyurethane topcoat (coating D); three layers of epoxy polyamide (coating G), and (c) IVD-A1 primer and epoxy polyamide topcoat (coating E); IVD-A1 primer, epoxy polyamide midcoat, polyurethane topcoat (coating F).

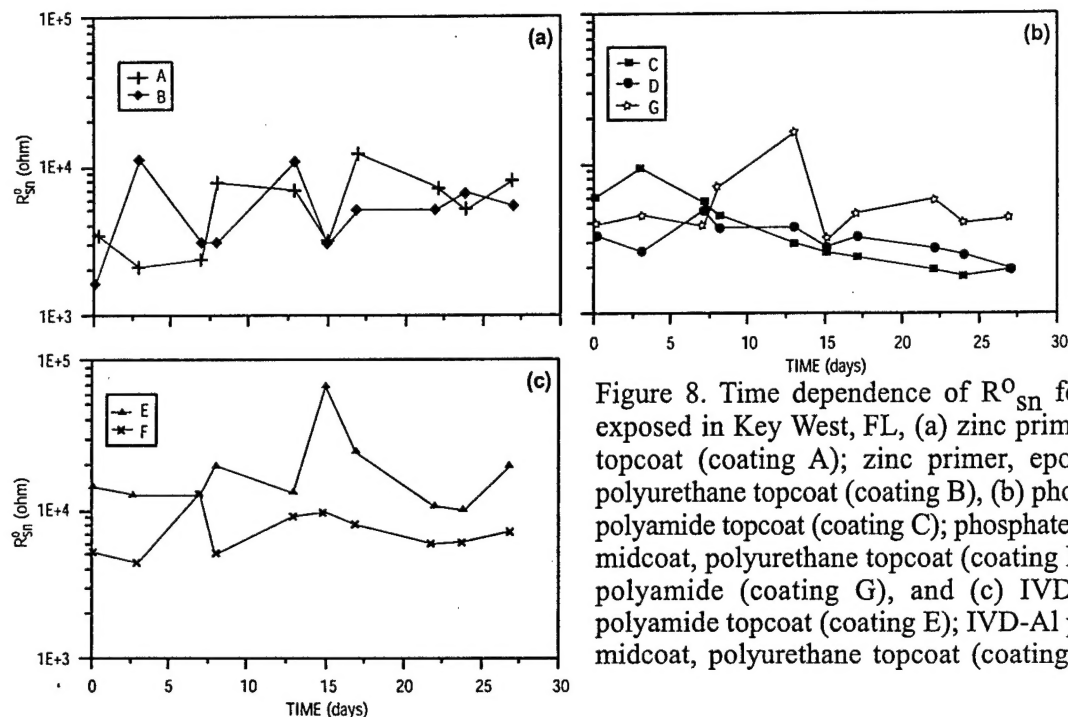


Figure 8. Time dependence of  $R^0_{sn}$  for coatings with defects exposed in Key West, FL, (a) zinc primer and epoxy polyamide topcoat (coating A); zinc primer, epoxy polyamide midcoat, polyurethane topcoat (coating B), (b) phosphate primer and epoxy polyamide topcoat (coating C); phosphate primer, epoxy polyamide midcoat, polyurethane topcoat (coating D); three layers of epoxy polyamide (coating G), and (c) IVD-Al primer and epoxy polyamide topcoat (coating E); IVD-Al primer, epoxy polyamide midcoat, polyurethane topcoat (coating F).

For samples with intentional defects coupled to a zinc electrode,  $R_n$  and  $R^0_{sn}$  data were obtained during immersion in artificial seawater for 30 days (Figure 9a-d).  $R_n$  and  $R^0_{sn}$  generally increased with time for all samples. These increases are most likely due to formation of calcareous deposits in defects that increase pore resistance ( $R_{po}$ ). Impedance spectra obtained during immersion in artificial seawater confirmed the assumption that  $R_{po}$  increased as defects filled with calcareous deposits. Spectra changed from a one-time constant mechanism at early exposure times to spectra containing the polymer coating capacitance ( $C_c$ ) at the highest frequencies and  $R_{po}$  at the lower frequencies where EN data are recorded (Figure 10a,b). In these cases,  $R_n$  and  $R^0_{sn}$  are related to  $R_{po}$  (5).

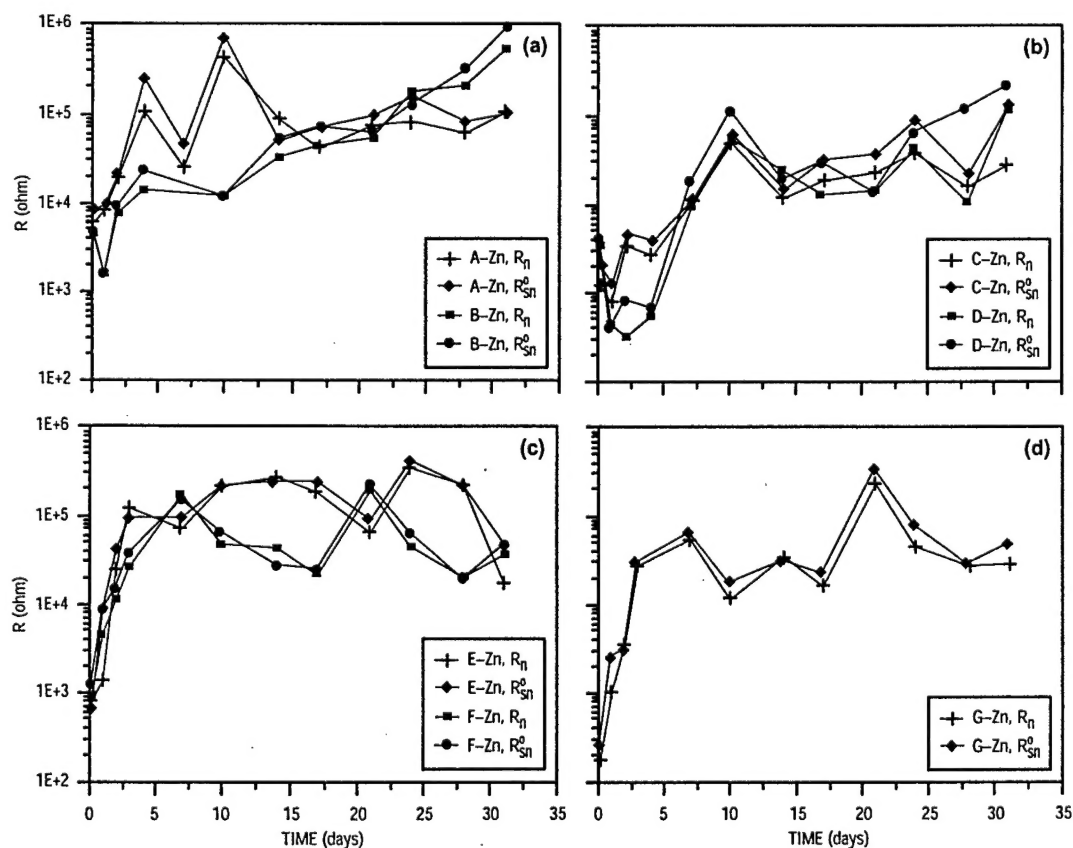


Figure 9. Time dependence of  $R_n$  and  $R^0_{sn}$  for cathodically protected coatings with defects exposed in artificial seawater, (a) zinc primer and epoxy polyamide topcoat (coating A); zinc primer, epoxy polyamide midcoat, polyurethane topcoat (coating B), (b) phosphate primer and epoxy polyamide topcoat (coating C); phosphate primer, epoxy polyamide midcoat, polyurethane topcoat (coating D), (c) IVD-Al primer and epoxy polyamide topcoat (coating E); IVD-Al primer, epoxy polyamide midcoat, polyurethane topcoat (coating F), and (d) three layers of epoxy polyamide (coating G).

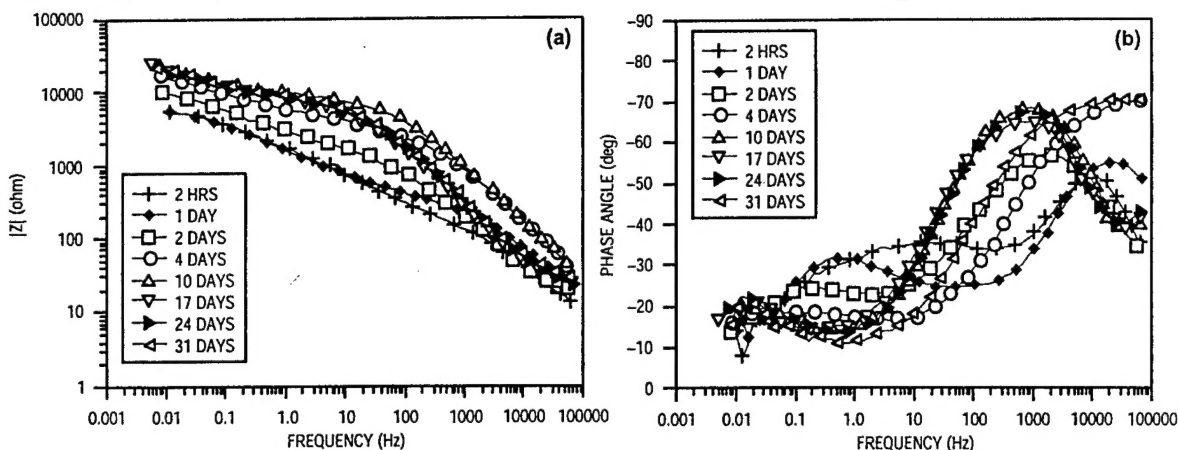


Figure 10. EIS spectra as a function of exposure time to artificial seawater for coating with IVD-Al primer and epoxy polyamide topcoat (coating E).

The product  $R_{sn}^0 \times I_{coup}$  had the same approximate value of 10 mV for all coating systems (Figure 11). This result is similar to the relationship between the corrosion current ( $I_{corr}$ ) and polarization resistance ( $R_p$ ) ( $I_{corr} = B/R_p$ ). The parameter  $B$  defined as  $B = (b_a \cdot b_c) / (2.3(b_a + b_c))$ , where  $b_a$  and  $b_c$  are the anodic and cathodic Tafel slopes, respectively, equals 10.4 mV for  $b_a = 120$  mV and  $b_c = 30$  mV.  $B = 10$  mV is very similar to the product of  $R_{sn}^0 \times I_{coup}$  from noise measurements (Figure 7). In principle,  $I_{coup}$  does not equal  $I_{corr}$  and  $R_{sn}^0$  equals  $R_p$  only for spectral noise plots that are independent of frequency (6).

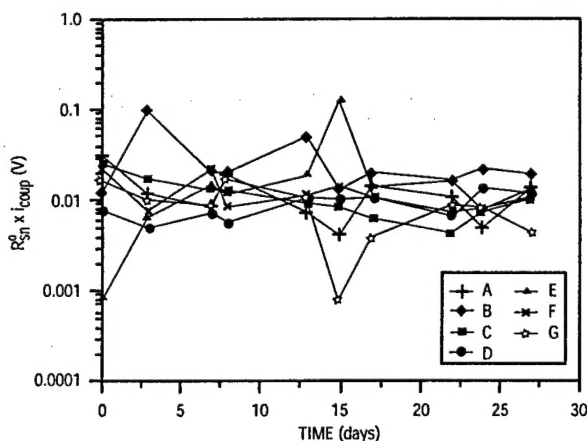


Figure 11. Time dependence of  $R_{sn}^0 \times I_{coup}$  for coatings with defects exposed in Key West, FL.

## Spatial Relationships Experiments

After a one hour exposure to established anodes on corroding iron filings, large numbers of viable bacteria were found in association with iron corrosion products in natural seawater, basal salts medium and in distilled water (Figure 12). The same observation was made with fixed cells.

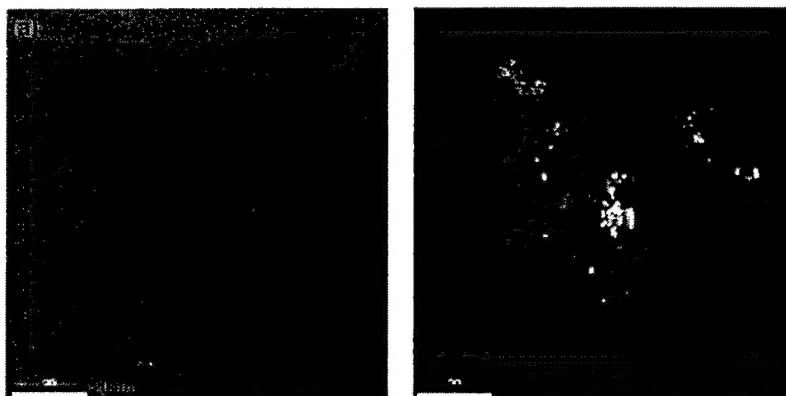


Figure 12. (a) Corroding area on iron filing (indicated by arrow) and (b) CLSM of bacteria attached preferentially at corroding area.





## DISCUSSION

In all marine exposures, localized corrosion of iron-containing substrata was accompanied by large concentrations of marine bacteria irrespective of alloy or coating. There were no indications of corrosion products within defects of coatings that had zinc primer with or without external anodes. In the presence of an anode, defects were filled with crystalline material that was predominantly calcium. Relative amounts of zinc and calcium varied. Furthermore, large concentrations of bacteria could not be detected within defect areas protected by either zinc as primer or as sacrificial anode. In all other cases, defects were mounded with bright reddish-orange corrosion products and large concentrations of marine bacteria. Tightly coiled helical shapes, typical of some iron-oxidizing bacteria, reportedly rare in the marine environment (7), were concentrated within the corrosion products on samples with phosphate primer, epoxy polyamide midcoat, and polyurethane topcoat. These organisms, in association with mounds or tubercles of iron corrosion products, are often used as indicators of MIC (8,9).

Franklin *et al.* (10) reported bacterial cells associated with anodic regions of carbon steel detected using scanning vibrating electrode microscopy. They were cautious not to interpret their data to indicate that the cells caused the anodic sites. They did observe that once bacteria were associated with anodic regions, those areas remained anodic for the duration of the experiments. Anodic sites not associated with bacteria were transient. Little *et al.* (11) made similar observations of spatial relationships between *Oceanospirillum* and anodic regions on copper surfaces. deSanchez and Schiffrin (12) demonstrated chemotaxis of a marine *Pseudomonas* sp. induced by transition metal ion concentration gradients from corroding copper and titanium surfaces. Loeb and Neihof (13) used microelectrophoresis to demonstrate that particles in the marine environment, including bacteria, are negatively charged and attracted to positive charge. The nature of the attraction identified in this paper has not been identified, but does not depend on cell viability or conductivity of the electrolyte.

## SUMMARY AND CONCLUSIONS

The distribution of bacteria on stainless and mild steel was strongly influenced by the presence of iron corrosion products. In natural marine exposures, accumulations of bacteria were consistently co-located with iron oxide corrosion products. When either a zinc primer or a zinc anode provided cathodic protection, defects were filled with calcareous deposits and contained only isolated bacterial cells, similar to areas of intact coating. The product  $R_{sn}^o I_{coup}$  had the same approximate value of 10 mV for all coating systems, suggesting that the mechanism of the corrosion reaction occurring in the intentional coating defects is very similar for all coating formulations.

## ACKNOWLEDGMENTS

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# SPATIAL RELATIONSHIPS BETWEEN MARINE BACTERIA AND LOCALIZED CORROSION ON STEELS

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Running title: Spatial Relationships

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